

## REMARKS

Applicants herein amend claims 1-5, 7-12 and 20; and add new claims 24-31. Claims 1-14 and 19-31 are pending upon entry of these amendments.

Claim 1 is amended to recite that the reaction mixture include “at least one of a fluorescent probe, beacon or intercalating dye” and that the reaction mixture is for primer-based amplification “and detection.” Support for this amendment can be found, for example, at paragraph 0038 of the published application which describes the use of fluorescence probes, beacons, and/or intercalating dyes for detection in quantitative PCR techniques.

New claim 31, relating to “an improved reaction mixture for the detection and/or quantification of a target nucleic acid”, similarly finds support in original claim 1 and paragraph 0038, as well as at paragraph 0025, which specifies that amplification reactions find use in nucleic acid detection and quantification methods. Dependent claims 2-5, 7-10 and 20 are amended merely to reflect dependency from new independent claim 31, as well as from independent claim 1.

Method claim 11 is amended to independent claim format and to include “detecting the amplicons” produced by “amplifying the target nucleic acid”. Support for this amendment can be found, for example, at paragraph 0042, which specifies that a preferred embodiment involves “amplification reactions that focus on detection of a product”; and at paragraph 0025, which discusses methods for detecting and quantifying the amount of amplicon(s) produced by the reaction. Further, Examples 1-3 of the specification indicate use of dUTP in PCR reactions where amplicons are detected and/or quantified.

Method claim 12 similarly is amended to independent claim format, to include “detecting the amplicons” and to recite “detecting and/or quantifying a target nucleic acid”. As discussed above, support for these amendments can be found in paragraphs 0042 (detection methods) and 0025 (detection of amplicons and use in quantification methods). Further, again as discussed above, Examples 1-3 of the specification indicate use of dUTP in PCR reactions where amplicons are detected and/or quantified. Accordingly, these amendments present no issues of new matter.

There is also no issue of new matter with respect to new claims 24-31. These claims depend from newly-independent claims 11 and 12 and track the subject

matter of dependent claims 2-10. Accordingly, they merely reflect dependencies previously presented in the multiple-dependencies of former claims 11 and 12.

With respect to the amended and new claims, Applicants have not dedicated or abandoned any unclaimed subject matter and moreover have not acquiesced to any rejections and/or objections made by the Patent Office. Applicants reserve the right to pursue prosecution of any presently excluded claim embodiments, for example, in future continuation and/or divisional applications.

#### **IDS, Corrected Drawings, Sequence Listing**

Applicants thank the Examiner for acknowledging the propriety of the IDS submission of December 11, 2008, and for acceptance of the corrected drawings of December 5, 2008. Action at page 2.

Nonetheless, Applicants note that the Examiner finds Figures 5A and 5B not in compliance with the sequence rules set forth in 37 CFR § 1.821-1.825. *Id.* Specifically, the Examiner requests submission of (a) a paper copy of the sequence listing; (b) the sequence listing in computer readable format (CRF); (c) a statement regarding the identical nature of the paper copy of the sequence listing and its CRF version; and (d) an amendment to the specification to identify the nucleotide sequences disclosed in Figures 5A and 5B in the section regarding the Brief Description of Drawings. Action at pages 2-3.

Applicants submit herewith a sequence listing (both paper and CRF versions), a statement of identity, and an amendment to the specification, as requested by the Examiner. Applicants respectfully submit that these submissions render Figures 5A and 5B in compliance with sequence rules 37 CFR § 1.821-1.825.

#### **Withdrawal of All Prior Objections and Rejections**

Applicants thank the Examiner for withdrawal of all prior objections and rejections based on Applicants' amendments and/or arguments of December 11, 2008. Action at pages 3-4. Applicants note, however, that the rejection of claims 5, 6, and 15-18 under 35 U.S.C. § 103(a) is withdrawn in view of re-application of the rejection, along with claims 1-4 and 7-12. Applicants respectfully submit that the instant amendments overcome the new rejections, as discussed below.

### **Rejections Under 35 U.S.C. § 103**

#### *Danielsen and Haberhausen references*

Claims 1-12 are rejected under 35 U.S.C. § 103(a) as being obvious in view of Danielsen et al., WO 02/090536 A2 (“Danielsen”) and further in view of Haberhausen et al. US 6,248,522 (“Haberhausen”). Action at pages 5-7. Specifically, the Office alleges that Danielson discloses a reaction mixture comprising dNTPs and dUTP, wherein the dUTP replaces about 25% of the dTTP and a range of 10-80% substitution. Action at page 5. The Office further alleges that it would have been obvious to one of ordinary skill in the art at the time the invention was made to use the U-container primers taught by Haberhausen in the procedures of Danielsen to arrive at the claimed invention. Action at pages 6-7.

Applicants respectfully submit that the current amendments to claim 1, from which claims 2-10 depend, and to claims 11 and 12, clearly overcome these rejections. Specifically, as noted above, amended claim 1 includes “at least one of a fluorescent probe, beacon or intercalating dye” with the dNTP/dUTP mixture; while claims 11 and 12 now require the additional step of “detecting the amplicons” resulting from the amplification step. Applicants respectfully submit that Danielsen fails to teach or suggest including “a fluorescent probe, beacon or intercalating dye” with the dNTP/dUTP amplification mixture, nor does it teach or suggest “detecting the amplicons” produced. Moreover, Haberhausen cannot properly be combined with Danielsen to remedy these deficiencies.

Danielsen is directed to generating randomly-sized nucleic acid fragments for shuffling to produce recombinant polypeptides encoding proteins with desired altered properties. Accordingly, the U-containing amplicons resulting from Danielson’s amplification reaction are purified and then deuracilated (e.g., using uracil-DNA-glycosylase) as a step to creating the randomly recombined polynucleotides. See, e.g., page 19, lines 1-15. For example, deuracilated product may be cleaved to give randomly-sized fragments, which subsequently are recombined and extended. See, e.g., page 23, lines 17-26.

Danielsen mentions the use of an “affinity label on either templates or primers” to aid bead purification of the U-containing amplicons (page 19, line 3), but nowhere teaches or suggests detecting the purified product; nor including a fluorescent probe, beacon or intercalating dye in the amplification mixture. Rather,

Danielsen specifies that the label is to provide “a simple means for separation”, and further states a preference for “labeled *templates*” (emphasis added), which is obviously contrary to detecting the resulting amplicons. See page 23, lines 6-8. Indeed, Danielsen discusses detection only in an entirely different context, namely the detection of enzymatic activity of the recombined polypeptides after multiple intervening steps. See, for example, page 26, lines 3-5, providing that “[t]he enzymatic activity may be detected by a dye, fluorescence, precipitation, pH indicator, IR-absorbance or any other known technique for detection of enzymatic activity.”

In contrast, the amplicons produced in accordance with the instant methods are directly detected without further manipulation since the incorporation of dUTP renders them subject to subsequent mutation (which Danielsen seeks to exploit but the instant approach seeks to avoid). Consider, for example, paragraph 0042 of the instant specification, which explicitly makes this distinction:

“Replacement of a portion of dTTP in a conventional dNTP mix in a primer-based amplification reaction with a combination of dUTP and at least one more unconventional nucleotide, for example dITP or deaza-dGTP. Note that this embodiment is most useful where incorporation of mutations into the amplicon is not a major issue, given that incorporation of ITP, or other non-dUTP like unconventional bases, into an amplicon can cause high mutation rates in the product, i.e., amplification reactions that focus on detection of a product and not the use or sequencing of the product are most preferred for this embodiment.” (Emphasis added)

Thus the detection methods recited in the subject claims are clearly distinguished from Danielson, which focuses exclusively on subsequent degradation and manipulation of the amplicons, for example, in using the U-containing amplicons to generate randomly-sized fragments that then can be recombined and extended, as discussed above. Moreover, the dUTP/dTTP ratios discussed in Danielsen are specifically directed to such use, as partial substitution of dTTP with dUTP leads to incorporation of uracil at random positions along a nucleic acid sequence. Indeed, one of ordinary skill in the art interested in detection of an amplification product would have no reasonable basis to turn to Danielsen, a reference that never discusses such detection.

Haberhausen cannot remedy the deficiencies of Danielson as the two references cannot properly be combined to arrive at the detection methods of the subject claims. Applicants respectfully submit that there would be no motivation to

combine the U-containing amplicons of Haberhausen with Danielsen, because detection of U-containing amplicons plays no part in Danielsen's procedures. As discussed in detail above, Danielsen's protocol employs U-containing amplicons exclusively to generate random-sized fragments in the production of recombinant proteins with altered properties, and provides no basis whatsoever to directly detect these amplicons. Accordingly, the deficiencies of Danielsen cannot be rectified by reliance on Haberhausen.

Applicants thus respectfully submit that there is no *prima facie* case for obviousness based on the above references, and earnestly and respectfully request reconsideration and withdrawal of the 103(a) rejections directed at claims 1-12.

Further, like amended claim 1, new claim 31 includes "at least one of a fluorescent probe, beacon or intercalating dye" in the reaction mixture, an element missing from Danielsen and not properly supplied by Haberhausen, as discussed above. Accordingly, Applicants respectfully submit that there is also no *prima facie* case with respect to claim 31.

#### *McLaughlin reference*

Claims 13, 14 and 19-23 are rejected under 35 U.S.C. § 103(a) as being obvious over Danielsen, in view of Haberhausen, as applied to claims 1-12 above, and further in view of McLaughlin et al. U.S. Patent No. 6,783,940 ("McLaughlin"). Action at pages 7-9. While acknowledging that neither Danielsen nor Haberhausen disclose sorbitol or manitol, the Office points to McLaughlin as teaching that sorbitol reduces non-specific amplification in PCR. Action at page 7. The Office contends that Danielsen's use of specific primers, along with McLaughlin's reference to dNTPs and dUTP, would lead one of ordinary skill in the art to employ McLaughlin's sorbitol in Danielsen's procedures. Action at pages 8-9.

Applicants respectfully point out that claims 13, 14 and 19 depend from independent claim 11; claims 20 and 21 depend from independent claims 1 and 31; and claims 22 and 23 depend from independent claim 12. As discussed above, Danielsen fails to teach one or more elements of each of the independent claims and neither Haberhausen nor McLaughlin can cure these deficiencies. Accordingly, there is likewise no *prima facie* case for obviousness based on Danielsen, Haberhausen and/or McLaughlin with respect to the dependent claims, and the same is true of newly-added dependent claims 24-30, that depend from claims 11

and 12. Accordingly, Applicants respectfully submit that there is no *prima facie* case for obviousness based on the cited references, and earnestly and respectfully request reconsideration and withdrawal of the 103(a) rejections directed at dependent claims 13, 14 and 19-23.

## CONCLUSION

Applicants respectfully submit that current claims 1-14 and 19-31 are now in condition for allowance. Applicants thus earnestly and respectfully request timely notification of same.

If a telephone call would help expedite any aspect of the prosecution of the instant application, Applicants encourage the Examiner to contact the undersigned by telephone at (415) 318-1200 or by fax at (415) 318-1300.

Respectfully submitted,  
KING & SPALDING LLP

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By: 

Todd A. Lorenz, Reg. No. 39,754

**Customer Number:** 504616  
King & Spalding LLP  
Four Embarcadero Center, Suite 3500  
San Francisco, CA 94111  
Telephone: (415) 318-1200  
Facsimile: (415) 318-1300